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Estrogen Response in Totally Depancreatized Female Dogs under Insulin Treatment

By

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CONTENT

I. Introduction

Abundant studies have been accumulated concerning the relationship between diabetes mellitus and hormone balance. Endocrine glands can maintain their normal function under superior control of the inter-brain and hypophysis, on the other hand under close cooperation between each of them.

Description of polyuria is found in literature as early as about 2000 years ago. It was THOMAS CAWLEY[1] who reported in 1788 for the first time close relation between diabetes mellitus and disturbance of pancreatic function. ROKITANSKY later reported that he found some pathological change of pancreas in 13 cases out of 30 autopsied cases of diabetics. In 1682, JOHANN CONREMD BRUNNER succeeded for the first time in total pancreatectomy in dogs, which was afterwards followed by MEHRING and MINKOWSKI[2] in 1889 and by DRAGSTEDT[3] in 1943, and the former reported the occurrence of characteristic symptoms of diabetes after total pancreatectomy. By this experiment, the existence of close relationship between diabetes and the pancreas has come to be unquestionable. In our country, total pancreatectomy was first performed by TSUCHIYA[4] in rabbits in 1924. Total pancreatectomy in man was first performed successfully by ROCKEY[5] in 1943. Since HONJO[6] has succeeded for the first time in our country in 1954 in total pancreatectomy in man, experimental studies have been accumulated by HONJO[6], YOSHIOKA[7], OHNO and others on the pathophysiology following total removal of this organ. In our clinic KANEDA[8] studied thyroid function under insulin treatment after total pancreatectomy and AIKAWA[9] also adrenal function in the similar condition. They concluded that thyroid and adrenal functions are declined after total pancreatectomy as was demonstrated by HASEGAWA[10] in anterior pituitary function.

As an important symptom of diabetes, a series of disturbance in the genital glands is pointed out such as genital dystrophy, and it has been said that in females incidence of diabetes increases after crimacterium. In these respects, it has been supposed that there must exist certain relationship between diabetes and the genital gland. However, very little has been clarified. Although there can be found some reports of clinicians that estrogen or testosterone is effective in diabetes, its mechanism is unknown. In 1954, HOUSSAY[11,12] reported that estrogen inhibits the occurrence of diabetes in rats when the pancreas is partially removed, while androgen enhances its occurrence.

On the other hand, YOSHIDA[13] reported that administration of androgen in diabetics frequently resulted in lowering of blood sugar level.

The author of the present paper made an experiment on the function of the genital gland and estrogen response under insulin treatment after total pancreatectomy in female dogs. Response of estrogen and 17-ketosteroid (abbreviated hereafter to 17-KS) were studied using chemical determination methods, at the same time blood sugar level, serum protein content, B. S. P. retention rate, body weight and weight of ovarium were determined. Histological study was also made on ovarium. Some new informations were ob-

tained and are to be reported.

II. Materials and Methods

1. Experimental Animals

Healthy adult mongrel female dogs weighing 8 to 13 kg were used.

2. Operative Procedure of Total Pancreatectomy

Operation was performed after the method of Markowitz[14]

Cocktelin H of 50 mg was intramuscularly administered in 2 injections with an interval of time of 30 minutes as a premedication in the fasting state. Isozol of 10 to 20 mg per kg body weight was intravenously administered. The abdomen was opened with upper median incision. The pancreas of normal dogs is consisted of the duodenal and free lobes, both of which are not fixed to the retroperitoneum. Total pancreatectomy was first set about by the ligation and severance of the branches of the pancreaticoduodenal vessels which enters the duodenal lobe. The duodenal lobe was isolated and freed from the duodenal wall. Then the free lobe was isolated by ligation of the branches of gastrolienal vessels and severance from the mesentery. Finally the pancreas was totally removed by dull isolation between the pancreas and the duodenal wall.

3. Collection of Urine

In collecting the urine for steroids determination, deliberate attention was paid for absolute separation of feces and urine.

Dogs were fed in a cage made of iron bars, having a bottom of duralumin plate with small holes like a net-work. A funnel of mild declivity made of steel plate was attached below the bottom and a bottle was placed under the funnel. Size of the cage was so large as not to cause stress and external stimuli were also shielded as possible.

4. Determination of Estrogen[15]

i. Equipments and Apparatus

Chromatographic vessels were made of glass with a diameter of 5 mm and 10 to 20 cm in length, having a spherical swelling of 10 to 20 ml in the upper portion and with a diameter of 1 mm in the end.

A set of ether vaporizing apparatus made of glass and separation funnel of 300 ml and 100 ml, and other common glass materials were used.

Colorimeter of Shimazu and Co. QB 50 was used, which enabled determination of longer waves than 400 mμ.

ii. Reagents and Solvents

Ether of special grade was vaporized and the fraction vaporized between 34° to 36°C was used. Quinol-H_2SO_4 reagent of 2 ml is added to the residue of vaporization of 20 ml of ether and heated. Here the tincture should not be stronger than in blank. Coarse ether is rinsed twice with 1/10 volume of 5 % solution of $FeSO_4$ in 2 % H_2SO_4, and further with 1/10 volume of 5 % sodium bicarbonate and common water and the fraction vaporized between 34° to 36 C with $CaCl_2$ dessication.

Benzene, HCl, NaOH and $NaHCO_3$ of special grade, H_2O_2 of 30 % of lst grade, boric acid of J. P. and $(CH_3)_2SO_4$ available in market were used.

Alumina of Brockmann was used.

Quinol-H_2SO_4 reagent was prepared by rapid solving of 2 g of quinol (hydroquinone)

of lst grade or photographic use with heat in 100ml of 65% (v/v) H_2SO_4 and stored in shadowed bottle.

Thirty per cent H_2SO_4 was prepared by adding 70 ml of water to conc. H_2SO_4 of 30 ml.

iii. Chromatographic solvent

Petroleum ether of special grade was vaporized and the fraction of 30° to 37°C was used.

Anhydrobenzene of special grade was added with metallic natrium in order to absorb water and revaporized for use.

iv. Determination

A. Hydrolysis, Extraction and Rinse.

When urine excretion is less than 1000 ml a day, urine was adjusted to 1000 ml with water, 200 ml of which was added with 30 ml of HCl and boiled for an hour for hydrolysis, steam-bath or reflux condenser being attached. After cooled down with ice, 100ml of ether is added and vigorously shaken in a separation funnel for 15 seconds. Inferior layer was further extracted with 100 ml of ether. The ether was added to 50 ml of 8% sodium bicarbonate solution and shaken and the inferior layer was discarded. Then it was well shaken with 15ml of 8% NaOH, and 60 ml of 8 % $NaHCO_3$ was added, vigorously shaken and the inferior layer was discarded. It was further shaken with 15 ml of 8 % $NaHCO_3$ and finally with 10 ml of water. The water was discarded and removed as possible. The ether was transfered to flask and vaporized until approximately 5 ml of ether remained.

B. Saponification

Benzene of 50 ml was added to the content of the flask for dilution and extraction was made twice with 25 ml of 4 % NaOH solution. Together with this 4 % NaOH solution, the content was heated for 30 minutes in boiling water, and 6 g of $NaHCO_3$ was added before cooled.

Extraction was twice performed with ether of 25ml after cooled out with ice, and rinsed with 10 ml of water. Extraction was again performed twice with 25 ml of 1.6% NaOH solution.

C. Methylation

Boric acid of 0.9 g was added to the above mentioned 1.6 % NaOH solution, and solved with heat, the ether being evaporated. When cooled to about 37°C, 1ml of $(CH_3)_2SO_4$ was added with a pipette and stirred with a magnetic stirrer or shaken in a separation funnel. As oil drops disappeared, 1 ml of $(CH_3)_2SO_4$ was added again and shaken and let stand for an hour. Ten ml of 20% NaOH solution and 2.5 ml of 30% H_2O_2 were added and let stand for 5 minutes. The content was transfered to separation funnel and extracted twice with 25 ml of benzene. The benzene was twice rinsed with 10 ml of water, the water was removed as possible and vaporized and dried.

D. Purification with Alumina

a. Determination as Total Estrogen

Brockmann alumina of II activity of 0.8 g was put in a chromatographic vessel of diameter of 5 mm with benzene. Above mentioned dried up material was solved in 2 ml of 10% methanol-benzene and poured into the alumina. The flask was twice rinsed with

2 ml of 10% methanol-benzene and the fluid was poured into the alumina. The filtrative solution was dried up and subjected to colorimetry of Quinol-Kober's method.

b. Colorimetry of Quinol-Kober's Method

Material purified with alumina was vaporized and dried. Two ml of 2% quinol of 65% vol. H_2SO_4 was added and put in boiling water. At the 5 th minute, the content was stirred and heated for 20 minutes. After cooling in water, 0.25 ml of water was added and heated for 5 minutes. The mixture was diluted with 2 ml of 30% vol. H_2SO_4 and colorimetrical absorbability E was determined against reagents' blank through the filters of 480 mμ, 515 mμ and 550 mμ. Absorbability was corrected by Allen's correction.

Standard material of 10 μg of each estrogen methyl ether was tinctured and corrected absorbability (S) of 1 μg was previously sought. The corrected absorbability was calculated from following formula;

$$\text{Corrected Absorbability} = E_{515m\mu} - (E_{480m\mu} + E_{550m\mu}) \times \frac{1}{2}$$

$$\text{Estrogen Excretion per Day}(\mu g) = \frac{\text{Corrected Absorb. of Urine}}{S} \times \frac{\text{Volume of Urine per Day}}{200} \times 0.95 \times \frac{100}{57}$$

By the way, $\frac{100}{57}$ represents average collection rate of Eo, Ed and Et, and 0.95 is a coefficient for correction of increased molecular weight of estrogen by methylation into that of free type.

5. Determination of 17-KS in Urine[16]

i. Purification and Preparation of Solvents and Reagents

A. Ether of special grade was vaporized and used.

B. H_2SO_4 of special grade

C. Formalin of J. P.

D. N-NaOH of lst grade of 40 g was solved in 1000 ml of water.

E. One per cent m-dinitrobenzene ethanol solution

Purified m-dinitrobenzene of 1 g was solved in ethanol of special grade to be 100 ml in all and stored in a shadowed bottle.

a. Purification of m-dinitrobenzene

Fifty g of m-dinitrobenzene available in market was solved with heat in 250 ml of pure ethanol, 25 g of active coal was added and further heated for 5 minutes. Before cooled down, it was filtrated and the filtrate was gradually cooled. Crystal precipitated was filtrated and dried in room temperature and stored in shadowed bottle. Melting point was 90 to 91°C.

F. Solution of 8N-KOH

KOH of special grade of 50g was solved in distilled water of 100 ml.

G. Ethanol solution of 80% for dilution

Ethanol of special grade, which does not contain aldehyde was diluted to be 80 % with water.

H. Standard solution of dehydroepiandrosterone

Dehydroepiandrosterone of 10 mg was solved in special grade ethanol and made 100ml

in a measuring flask. Solution of 100 μg/ml.

ii. Determination of 17-KS.

A. Hydrolysis and Extraction for Determination of Total 17-KS

Volume of urine within 24 hours was determined, 10 ml of which was put in a test tube and 0.2 ml of 5 times diluted formalin solution and 1 ml of H_2SO_4 were added. Then it was heated for 15 minutes in steam-bath or boiling water for hydrolysis and cooled. The hydrolysed urine was put in a separation funnel of 50 to 100 ml, 15 ml of ether was added, shaken and separated. Together with the ether, it was put in another separation funnel, shaken two times with 5 ml of 4% NaOH, rinsed and layer of NaOH was discarded. Layer of ether was thrice shaken with 10 ml of water and rinsed until the reaction becomes neutral. Anhydrous Na_2SO_4 of about 2 g was added to the ether layer and dried. After filtrated through cotton stopper, Na_2SO_4 was rinsed with some ether and vaporized until dried out together with ether.

B. Determination by Zimmermann Reaction

Standard solution of dehydroepiandrosterone (100 μg/ml ethanol solution) of 0.5 ml was previously put in a series of test tubes and dried for standard material. To each of empty 2 test tubes for reagents' blank, 2 other test tubes of standard material and 1 more test tube of dried material for the determination, 0.4 ml of 1% m-dinitrobenzene ethanol solution was added to solve dried materials in the test tubes. KOH solution of 8 N of 0.2 ml was added, well mixed and let stand in the dark in room temperature, for 30 minutes in summer and an hour in winter for satisfactory result. The mixture was diluted with 4 ml ethanol of 80 % and colorimetrical absorbability of standard and referred material were determined through the filters of 460 mμ, 520 mμ and 580 mμ taking reagents' blank as control.

C. Calculation

Correction formula of Allen was applied and corrected value was obtained. Amount of 17-KS in the material was sought as the amount of dehydroepiandrosterone.

Correction Formula of Allen:

$$E_1 = E520m\mu - (E460m\mu + E580m\mu) \times \frac{1}{2}$$

Whereby E represents absorbability at the corresponding wave length, E_1 corrected absorbability.

Amount of 17-KS in urine of 24 hours was calculated from following formula.

$$\text{17-KS in Urine per Day (mg)} = \frac{P_{E_1}}{S_{E_1}} \times \frac{V}{v} \times 0.05$$

Whereby V represents volume of urine per day, v volume of urine used for the determination, P_{E_1} corrected value of the material and S_{E_1} corrected value of dehydroepiandrosterone of 50 μg (0.05 mg).

6. Determination of Blood Sugar Level

Somogyi-Nelson's[17] method was followed.

7. Determination of Serum Protein Content

Refractometer of Hitachi Co. was used.

8. Determination of B. S. P. Retention Rate

Bromsulphalein solution of 5 % was intravenously injected from the femoral vein in

a proportion of 0.1 ml per kg body weight. Determination was performed 15 minutes later.

9. Histological Studies

Hematoxylin-eosin double staining was employed for the study.

III. Results

1. Total Estrogen Excretion in Urine

a. Total Estrogen Excretion in Urine in Normal Female Dogs

Determination of estrogen in normal female dogs resulted in a data scattered within the range of technical error due to extremely minute estrogen excretion. Hence, estrogen determination was performed after previous administration of gonadotropin in normal dogs, that is, FSH serotropin of 1000 u. was intramuscularly injected once a day for 2 days. After that LH propogonil of 300 u. was intramuscularly injected once a day for 2 days, and the dog was put in the cage for urine collection for 48 hours. Estrogen in 48 hours' urine was determined, as represented in Tab. 1. By this previous injection, determination of estrogen became feasible.

Tab. 1. Estrogen Level in Normal Female Dogs.

Dog No.	Body Weight (kg)	GTH. non-treated		GHH. treated	
		Urine Volume mℓ	Estrogen μg/48th	Urine Volume mℓ	Estrogen μg/48h
1	9.0	720	(—	980	7.3
2	10.0	910	—	880	6.5
3	13.0	970	0.65	1190	10.1
4	9.5	760	—	1010	8.5
5	8.0	690	—)	970	9.3
6	11.0	1010	(—)	910	8.3
7	9.0	860	1.00	1310	11.2
8	12.0	930	(—)	860	7.7
9	8.0	950	0.30	1210	9.6
10	10.5	1020	(—)	980	7.3
Mean					8.5

Estrogen excretion in 10 animals of gonadotropin administration was maximum of 11.2 μg/48 hours and minimum of 6.5 μg/48 hours, average value being 8.5 μg/48 hours. Concerning fluctuation of estrogen excretion depending upon body weight or urine volume, certain decisive correlation could not be found, although number of cases was not sufficient to acquire some conclusion.

It was studied, how many days after the administration of gonadotropin, estrogen level returns to that before administration of gonadotropin. As is shown in Tab. 2, towards the end of 3rd week estrogen level returned to that before gonadotropin injection and was included within the range of technical error in all the cases.

Estrogen excretion a week after gonadotropin injection decreased to approximately a half level compared with that immediately after the administration. Two weeks after the administration, effect of gonadotropin already disappeared in dogs of No. 13 and No. 15. Three weeks after the administration, estrogen excretion returned to the level before the administration.

For the comparative study with totally depancreatized dog, gonadotropin was administered in normal dogs repeatedly, that is, 2 nd injection towards 4 th week, 3 rd injection towards 8 th week and 4 th injection towards 12 th week and estrogen excretion was determined at each period. As is represented in Tab. 3 and Fig. 1, it was observed that

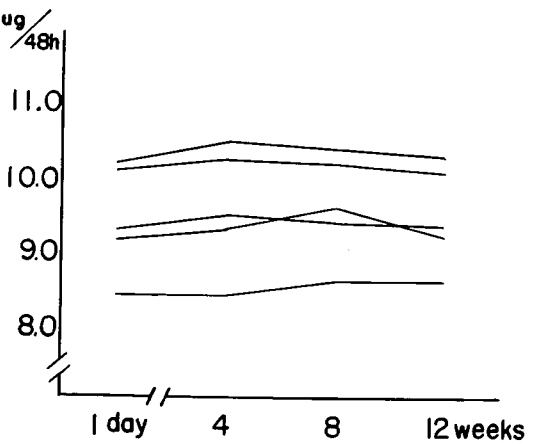
Tab. 2. Estrogen Level in Normal Female Dogs 1, 2 and 3 Weeks Respectively after Single Administration of Gonadotropin.

Dog No.	GTH. non-treated		G T H. treated							
			2 day		1th week		2th week		3th week	
	Urine Volume (mℓ)	Estrogen (μg/48h)	Urine Volume (mℓ)	Estrogen (μg/48h)	Urine Volume (mℓ)	Estrogen (μg/48h)	Urine Volume (mℓ)	Estrogen (μg/48h)	Urine Volume (mℓ)	Estrogen (μg/48h)
11	1240	(−)	1200	10.4	710	5.5	820	1.0	810	(−
12	1120	(−)	1170	9.7	810	4.6	760	0.3	720	(−)
13	840	(−)	880	8.5	790	4.0	840	(−)	830	(−)
14	1160	−)	1080	8.9	680	6.0	750	0.6	770	(−)
15	870	−)	720	7.0	810	1.8	810	(−)	780	(−)

Tab. 3. Estrogen Level in Normal Female Dogs after 1 st Gonadotropin Administration. 2 nd Administration at 4 th week, 3 rd and 4th Administration at 8th and 12th week.

Dog No.	2 day		4th week		8th week		12th week	
	Urine Volume (mℓ)	Estrogen (μg/48h)	Urine Volume (mℓ)	Estrogen (μg/48h)	Urine Volume (mℓ)	Estrogen (μg/48h)	Urine Volume (mℓ)	Estrogen (μg/48h)
16	840	9.3	860	9.5	880	9.4	850	9.3
17	810	8.4	820	8.4	830	8.6	830	8.6
18	870	10.2	850	10.5	890	10.4	840	10.3
19	830	9.2	860	9.3	850	9.6	820	9.2
20	920	10.1	920	10.3	840	10.2	890	10.1

Fig. 1. Estrogen Level in Normal Female Dogs after 1st Gonadotropin Administration, 2nd Administration at 4th week, 3rd and 4th Administration at 8th and 12th week.



and the decrease was gradual after 4th week. What was especially remarkable was that No. 33 dog, which showed conspicuous decrease in estrogen excretion at 4 th week and estrogen excretion remained in the similar level or slightly increased after 2nd administration compared with that after 1st injection. However, there was no case which showed less excretion than that after 1st administration of gonadotropin.

b. Total Estrogen Excretion in Urine in Totally Depancreatized Female Dogs

Total estrogen excretion in urine in totally depancreatized female dogs showed decrease in all the 10 cases, as shown in Tab. 4 and Fig. 2.

Decreased value was on the average 6.0 μg/48 hours at 4 th week, 5.6μg/48 hours at 8 th week and 5.2μg/48 hours at 12th week. Particularly, rapid decrease in estrogen was observed before 4th week

Tab. 4 Estrogen Level in Totally Depancreatized Female Dogs after Gonadotropin Administration

Dog No.	Before ope.		After Operation					
			4th week		8th week		12th week	
	Urine Volume (mℓ)	Estrogen μg/48h	Urine Volume (mℓ)	Estrogen μg/48h	Urine Volume (mℓ)	Estrogen μg/48h	Urine Volume mℓ	Estrogen μg/48h
25	920	10.6	1800	8.6	1830	7.1	1790	6.8
26	1140	13.5	1890	8.1	1760	6.9	1880	6.0
27	1000	10.6	1840	7.1	1870	6.4	2110	6.1
28	820	9.6	1640	6.0	1850	4.1	1920	3.6
29	960	10.5	1820	5.8	1790	4.6	1960	3.9
30	870	9.4	1800	6.6	1840	6.0		
32	1210	11.5	1920	6.4	1890	3.8		
33	770	8.4	1910	3.2				
34	860	12.3	1790	7.8	1960	6.5	1880	5.8
35	970	9.7	1810	6.4	1920	5.7	1940	4.7
Mean		10.5		6.0		5.6		5.2

Tab. 5 17-KS Excretion in Normal Female Dogs after Gonadotropin Administration.

Dog No.	2 day		4th week		8th week		12th week	
	Urine Volume mℓ	17-KS mg/48h	Urine Volume (mℓ)	17-KS mg/48h	Urine Volume (mℓ)	17-KS mg/48h	Urine Volume mℓ	17-KS mg/48h
16	840	0.83	860	0.85	880	0.86	850	0.93
17	810	0.98	820	0.98	830	0.98	830	1.02
18	870	1.02	850	1.04	890	1.08	840	1.08
19	830	0.98	860	1.00	850	1.01	820	1.03
20	920	1.05	920	1.06	840	1.09	890	1.12

Tab. 6. 17-KS Excretion in Totally Depancreatized Female Dogs after Gonadotropin Administration.

Dog No.	Before Ope.		After Operation					
			4th week		8th week		12th week	
	Urine Volume (mℓ)	17-KS mg/48h	Urine Volume mℓ	17-KS mg/48h	Urine Volume mℓ	17-KS mg/48h	Urine Volume (mℓ)	17-KS mg/48h
25	920	0.92	1800	1.23	1830	1.00	1790	0.62
26	1140	0.97	1890	1.50	1760	0.90	1880	0.83
27	1000	0.84	1840	1.20	1870	0.75	2110	0.36
28	820	1.00	1640	0.78	1850	1.00	1920	0.62
29	960	1.00	1820	1.62	1790	1.26	1960	0.78
30	870	0.63	1800	1.70	1840	0.56		
32	1210	0.90	1920	1.23	1890	1.00		
33	770	0.79	1910	1.14				
34	860	1.20	1790	1.11	1960	1.13	1880	0.83
35	970	1.14	1810	1.33	1920	0.97	1940	0.63
Mean		0.92		1.32		0.93		0.66

Fig. 2. Estrogen Excretion in Totally Depancreatized Female Dogs after Gonadotropin Administration.

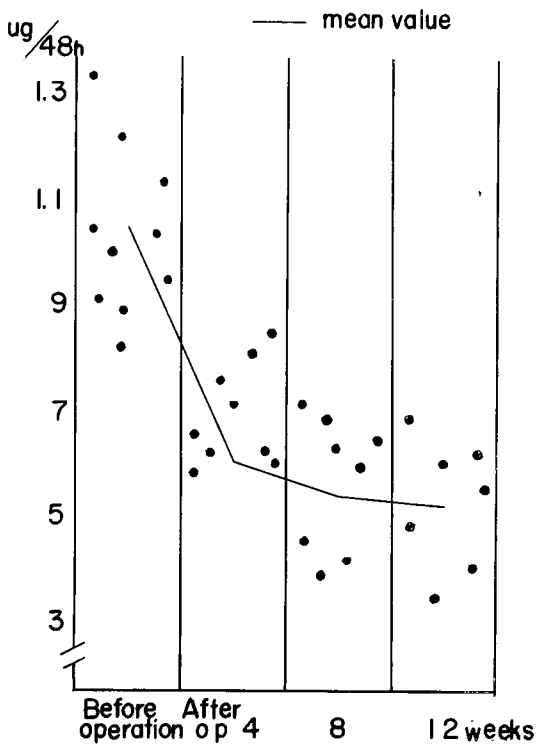
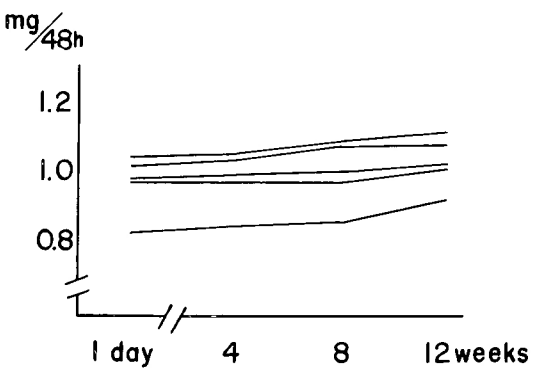


Fig. 3. 17-KS Excretion in Normal Female Dogs after Gonadotropin Administration.



showed weight loss of 0.9 kg until 4 th week, showed rapid weight loss after 4th week and died at 6th week, body weight at death being 5.2kg compared with that of 8.5 kg before total pancreatectomy.

2. Total 17-KS Excretion in Urine

a. Total 17-KS Excretion in Urine in Normal Female Dogs Treated with Gonadotropin

Gonadotropin was injected repeatedly in normal female dogs, 2nd injection towards 4th week after initial injection, 3rd injection towards 8th week and 4th injection towards 12th week and 17-KS excretion was determined at each period. As is shown in Tab. 5 and Fig. 3, 17-KS excretion showed slightly high level at 4th and 8th week, which yet increased slightly from 8th to 12th week. Decrease in 17-KS excretion was observed in no case.

b. Total 17-KS Excretion in Totally Depancreatized Female Dogs Treated with Gonadotropin

Gonadotropin was injected repeatedly in totally depancreatized female dogs in the same manner as in normal female dogs, and 17-KS excretion was determined at each period. Total 17-KS excretion increased by 0.4mg on the average 4 weeks after the operation compared with the level before the operation, which, however, decreased gradually from 8 th week to 12th week. The excretion level was 0.26mg lower on the average compared with that before the operation. When compared with 17-KS level in normal dogs treated with gonadotropin, the level was obviously decreased towards 8th to 12th week in totally depancreatized dogs, while in normal dogs the excretion increased at this period.

3. Blood Sugar Level

Insulin of 2 u/kg body weight was intramuscularly injected every day, and the administration was performed from intravenous route at the time of determination of blood sugar level.

Fig. 4. 17-KS Excretion in Totally Depancreatized Female Dogs after Gonadotropin Administration.

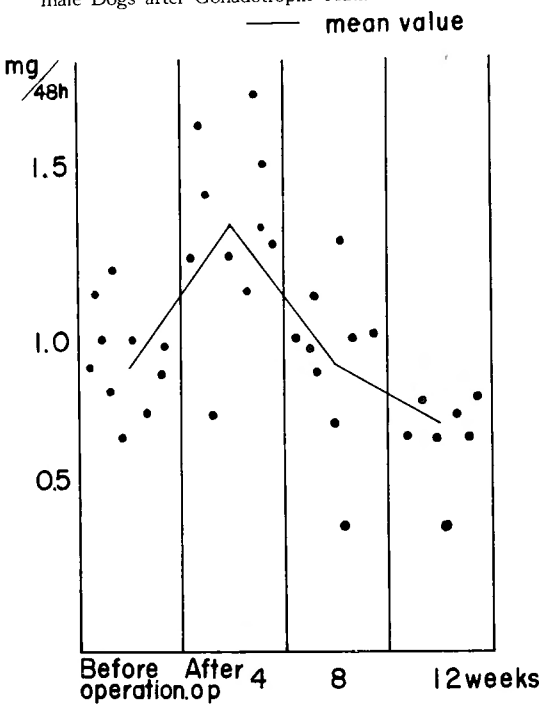
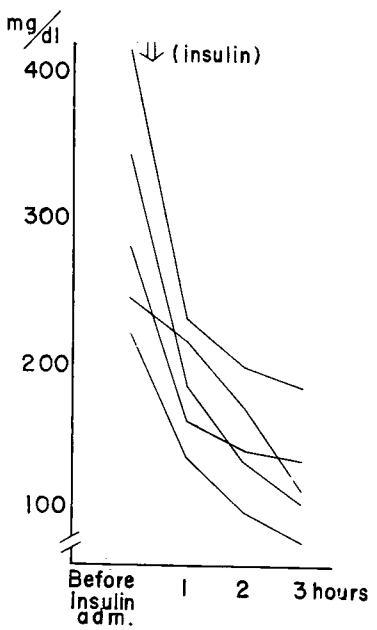


Fig. 5. Blood Sugar Level in Totally Depancreatized Female Dogs with Insulin Treatment and without Gonadotropin Administration.



Tab. 7. Blood Sugar Level in Totally Depancreatized Female Dogs with Insulin Treatment and without Gonadotropin Administration.

Dog No.	Before insulin administr. (mg/dℓ)	Hours after insulin administr.		
		1	2	3
21	280	160	138	133
22	246	213	169	113
23	342	183	131	102
24	220	135	98	78
25	416	231	198	183
Mean	300	164	146	121

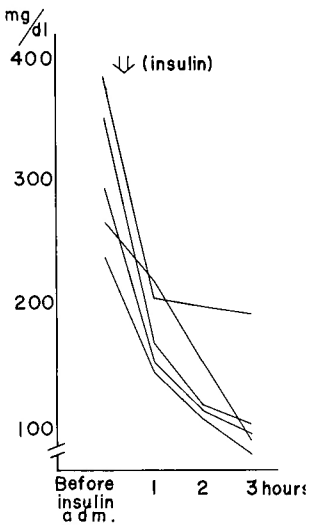
Tab. 8. Blood Sugar Level in Totally Depancreatized Female Dogs with Insulin Treatment and Gonadotropin Administration.

Dog No.	Before insulin administr. mg/dℓ	Hours after insulin administr.		
		1	2	3
26	292	150	110	91
27	236	143	104	74
28	264	214	151	87
29	350	166	112	97
30	384	201	195	190
Mean	305	154	134	107

Tab. 9. Serum Protein Content in Totally Depancreatized Female Dogs without Gonadotropin Administration.

Dog No.	Before ope. g/dℓ	After Operation.	
		4th week	8th week
21	7.8	5.5	
22	8.1	5.4	4.8
23	7.2	4.8	
24	7.3	6.2	4.7
25	7.1	5.1	
Mean	7.5	5.5	4.75

Fig. 6. Blood Sugar Level in Totally Depancreatized Female Dogs with Insulin Treatment and Gonadotropin Administration.

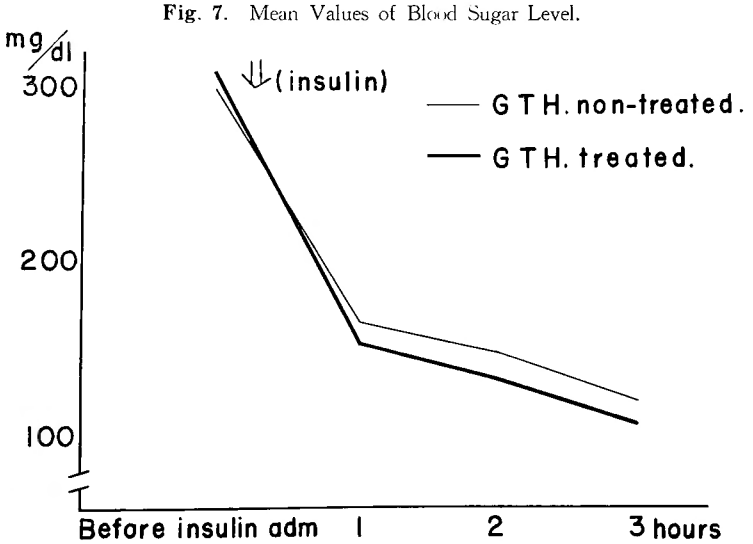


a. Blood Sugar Level in Totally Depancreatized Dogs Treated with Insulin and without administration of Gonadotropin.

Four weeks after total pancreatectomy, insulin was solely injected in the fasting state in the early morning, and blood sugar level was determined before insulin administration, an hour, 2 hours and 3 hours after it. Data obtained are summarized in Tab. 7 and Fig. 5.

Blood sugar level before insulin administration was 220mg/dl to 416mg/dl, 300 mg/dl on the average. One hour after the administration, blood sugar level decreased to 164 mg/dl on the average, which is approximately a half level of the previous one. Blood sugar level decreased on from 2 hours to 3 hours after

Fig. 7. Mean Values of Blood Sugar Level.



the administration, and reached the minimal value 3 hours after the administration which showed gradual tendency of increase thereafter.

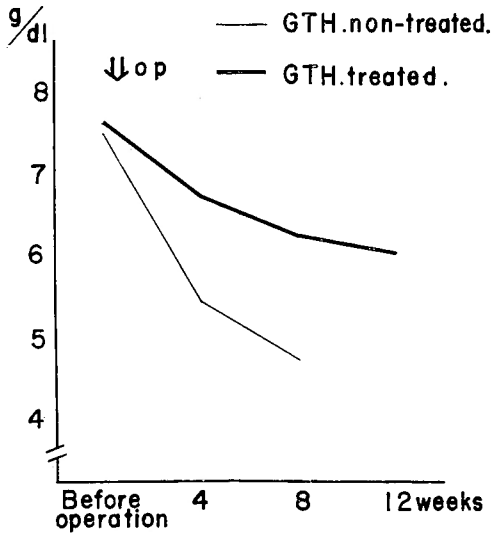
b. Blood Sugar Level in Totally Depancreatized Dogs Treated with Both Insulin and Gonadotropin

Two days after gonadotropin injection, blood sugar level was determined after insulin injection in the fasting state in the early morning. Blood sugar level decreased as in Tab. 8 and Fig. 6. Decrease in blood sugar level was more pronounced on the average in animals with gonadotropin administration compared with that in animals without the

Tab. 10. Serum Protein Content in Totally Depancreatized Female Dogs without Gonadotropin Administration.

Dog No.	Before Ope. (g/dl)	After Operation		
		4th week	8th week	12th week
25	7.7	6.8	6.8	6.4
26	7.4	6.4	6.4	6.2
27	8.1	7.0	6.2	6.0
28	8.2	7.2	6.4	6.1
29	7.1	6.5	6.1	5.9
30	7.0	5.7	5.3	
32	7.5	6.8	6.1	
33	7.2	5.1		
34	8.4	7.6	7.1	6.7
35	7.6	6.7	6.2	6.0
Mean	7.6	6.5	6.2	6.1

Fig. 8. Mean Values of Serum Protein Content.



Tab. 11. Body Weight of Totally Depancreatized Female Dogs without Gonadotropin Administration.

Dog No.	Before Ope.	After Operation	
		4th week	8th week
21	8.5	5.0	
22	12.0	9.0	7.0
23	9.5	5.4	
24	10.5	8.3	6.5
25	9.5	6.5	
Mean	10.0	6.8	6.75

Tab. 12. Body Weight of Totally Depancreatized Female Dogs with Gonadotropin Administration.

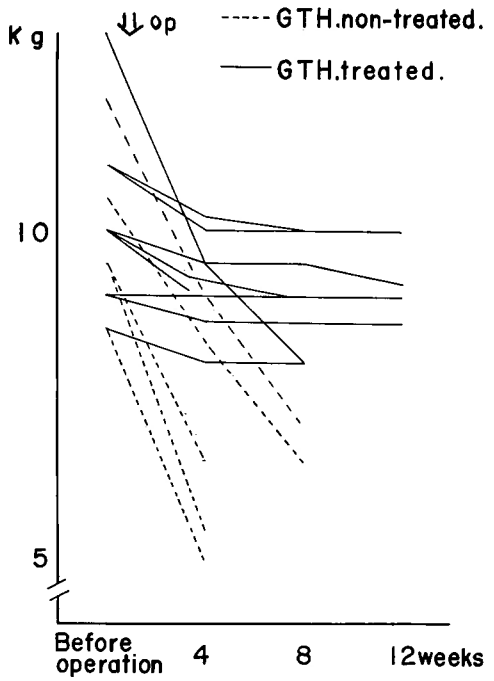
Dog No.	Before Ope. (kg)	After Operation		
		4th week	8th week	12th week
25	10	9.5	9.5	9.2
26	11	10	10	10
27	11	10	10	10
28	9	9	9	9
29	9	8.6	8.6	8.6
30	13	9.5	8	
32	8.5	8	8	
33	10	9.1		
34	10	9.3	9.0	9.0
35	11	10.2	10.	10.
Mean	10.2	9.3	9.0	9.4

administration. As is shown in Fig. 7, decrease was 10 mg/dl an hour later, 12 mg/dl 2 hours later and 14 mg/dl later and fall of blood sugar level was more prominent in animals with gonadotropin administration than in those with insulin administration alone. Shock frequently occurred in the period of simultaneous administration of insulin and gonadotropin.

4. Serum Protein Content

Serum protein content in totally depancreatized dogs without gonadotropin administration was 5.5g/dl on the average 4 weeks after the operation, as shown in Tab. 9, which is lower than that of 12th week in animals with gonadotropin administration. Serum protein further decreased to 4.75g/dl 8 weeks after the operation and some of the animals

Fig. 9. Fluctuation in Body Weight.



died at this period. Continuous determination was carried out until 12th week in no case. On the contrary, serum protein content did not decrease in animals with gonadotropin administration, as is shown in Tab. 10 and Fig. 8. Average decrease in serum protein until 4th week was 1.1g/dl. Decrease in serum protein in this group was as slight as 0.1 to 0.2g/dl 8 and 12 weeks after the operation.

5. B. S. P. Retention Rate

In B. S. P. retention test marked difference was not observed, the rate being less than 2.5% at 15th minute in both with and without gonadotropin administration.

6. Body Weight

Body weight of animals without gonadotropin administration is represented in Tab. 11. Marked weight loss of about a half of preoperative average of 10 kg was observed, postoperative weight being 6.8 kg 4 weeks after the operation. Few animals survived till 8th to 12th week. On the contrary, weight loss was slight in animals with gonadotropin administration, as shown in Tab. 12 and Fig. 9, average weight loss until 4th week was 0.9kg. Later than 4th week, weight loss was not observed except in 3 cases. Weight loss in these 3 cases, No. 30, No. 34 and No. 35 dogs, was also extremely slight.

Tab. 13. Change in Ovarial Weight.

Control				Totally depancreatized dog				
Dog No.	Body Weight (kg)	Ovary Weight (mg)	Ratio of Ovary Weight to body weight (mg/kg)	Dog No.	Body Weight (kg)	Ovary Weight (mg)	Ratio of Ovary weight to body weight (mg/kg)	Days after Ope.
1	9.0	1100	155.5	25	10.0	1120	142.0	105
2	10.0	1520	152.0	26	11.0	1600	115.1	91
3	13.0	1730	133.0	27	11.0	1200	109.0	101
4	9.5	1290	135.7	28	9.0	500	55.0	151
5	8.0	980	122.5	29	9.0	560	62.2	134
6	11.0	1590	111.5	30	13.0	1900	146.1	80
7	9.0	1180	131.1	32	8.5	800	94.1	78
8	12.5	1790	143.2	33	8.5	1080	127.0	38
9	8.0	1050	131.2	34	10.0	1210	124.0	122
10	10.0	1420	142.0	35	11.0	1520	138.1	113
Mean	10.0	1381	139.0		10.1	1182	114.3	

7. Weight of Ovarium

Weight of ovarium in control dogs was 1381 mg on the average and proportion to body weight was 139.0mg/kg, as is shown in Tab. 13. Ovarial weight in totally depancreatized dogs with gonadotropin administration decreased compared with control dogs, each data being 1182 mg and 114.3 mg/kg, respectively.

8. Histological Study of Ovarium

Ovarium extirpated 101 days (Plate 2) and 158 days (Plate 3) after total pancreatectomy showed the picture of atresia folliculi and marked proliferation of interstitial tissue, suggesting functional decay of the organ compared with that of normal dogs (Plate 1).

IV. Discussion

Concerning the function of genital glands after total pancreatectomy, no elaborate report is published yet. Studies of this aspect have been carried out histologically. In 1924, TSUCHIYA studied in rabbits the change of parotid gland and genital gland after total pancreatectomy and reported that parenchyma of the genital gland is likely to show atrophic changes and degeneration, whereas interstitial tissue generally shows hyperplasia. In 1954, YOSHIOKA observed a picture of atresia folliculi and interstitial hyperplasia in the ovarium of totally depancreatized dogs. Investigations on the change of genital gland after total pancreatectomy have been carried out at most to this extent, and there is no report on biochemical determination of estrogen. This is partly due to the fact that the method of determination itself is difficult and a plenty amount of urine is required for it. In 1954, YOSHIOKA carried out biochemical determination of estrogen after total pancreatectomy using ALLENDOISY method and reported decrease in estrogen.

The author of the present experiment carried out determination of estrogen in normal female dogs in the aim of exploring the function of the genital gland after total pancreatectomy. Biochemical method of estrogen determination has been investigated by many researchers in parallel with the studies on biological assay. However, biochemical determination of estrogen has been confronted to great difficulty owing to extremely minute excretion in urine compared with other hormones and the problem of urochrom which interferes the colorimetry. Extraction of estrogen from urine of man was first attempted by COHEN and MARRIAN[18)19)] in 1934, which was followed by studies of SMITH and SMITH[20)] and others. Principle of biochemical determination of estrogen is as follows ; most part of estrogen exists in urine in a type combined with glucuronic acid and sulfric acid and is water soluble, which becomes isolated type by hydrolysis.[21,22,23,24)] Then estrogen is extracted by some method[25,26,27)] utilizing characteristics of it. Extraction is performed first with mild alkali and then intense alkali, which is based on the difference in characters between intense phenol fraction most soluble in mild alkali of pH 8 to 11 and mild phenol fraction most soluble in NaOH solution of 0.1 to 1N. Dissolution chromatography is applied to this extracts and estrogen is determined by some biochemical process[28,29,30)]. The author of the present paper carried out the determination following the method of KAMBEGAWA. No literature has been found concerning biochemical determination of estrogen in dogs. Results of total estrogen determination in urine in the present experiment obtained by above mentioned method came to accordance with those of other researchers. However, estrogen determination in normal female dogs resulted in data scattered within

the range of error owing to the fact that estrus comes only twice a year in dogs. Hence, estrogen determination was carried out by bringing forth the sexual cycle by gonadotropin administration.

Estrogen could be determined to be 8.5μg on the average in urine of 48 hours, as is shown in Tab. 1. Then, it was studied how many days later estrogen level returns to that before gonadotropin administration, when it is administered in normal female dogs. As is represented in Tab. 2, it was observed that estrogen level returns after 3 weeks to that before gonadotropin administration. Effect of gonadotropin administration almost disappeared after 2 weeks and it is assumed that condition of the organism completely returns after 3 weeks to that before the administration. There is a report of UKAI[31] and others that there are 2 types in urinary estrogen response against gonadotropin treatment, that is, one shows marked change within 2 days and another shows marked change later than 3 days, and most cases belong to the former. As the result in the present experiment corresponded to this, estrogen determination was performed for 2 days immediately after gonadotropin administration. However, it was observed that the effect of gonadotropin treatment disappears completely towards the end of 2nd week. When gonadotropin was administered repeatedly 4, 8 and 12 weeks after 1st administration, estrogen was in the same level as the determination at 1st administration or slightly increased level, as in Fig. 1. However, there was no case which showed lower level than that before lst gonadotropin administration. When gonadotropin was administered in totally depancreatized dogs, estrogen level showed rapid fall until 4th week which was followed by further gradual fall later than 8th week. Activity of the genital gland after total pancreatectomy showed rapid decay by 4th week and lowering of the function after 4th week was slight.

Loss of insulin and glucagon caused by total pancreatectomy affects not only nutritional absorption but endocrinological function of the inter-brain, anterior pituitary, thyroid and genital glands. In 1954, HONJO and HASEGAWA studied the response of the anterior pituitary after total pancreatectomy, particularly histological appearance of this organ which has the closest relation to glucose metabolism together with the pancreas, in which he calculated the proportion of each type of chromophile and chromophobe cells in the anterior pituitary by CRESAZAN's staining. They observed marked decrease in chromophile cells which have endocrine function and also similar decrease in basophilic and eosinophilic cells compared with normal dogs, suggesting decay of endocrine function of the anterior pituitary. KANEDA reported lowered thyroid function after total pancreatectomy which is presumably due to hypofunction of the anterior pituitary. GOTOH[32] reported that there exists hypofunction of the genital gland under thyroid hypofunction.

From these observations, it is assumed that function of the genital gland comes to decay after total pancreatectomy and the results of the present experiment also corresponded to these.

As an indicator of function of the adrenal cortex and genital gland, 17-KS is excreted in urine. This steroidketon shows bright violet color reaction when treated with m-dinitrobenzene and alkali, basis of which was discovered by VON BITTO and later applied to analysis of 17-KS by ZIMMERMANN[33]. ALLEN[34] later established correcting formula in which the data is corrected by the difference between colorimetric absorption at 520 mμ, at which absorption curve shows its peak and mean absorbability of 460mμ and 580mμ, i. e. 60mμ

above and below 520mμ of maximum absorption. Method of 17-KS determination made an advancement thereafter. Among these several methods[35,36,37], the author carried out the determination of following the method of KAMBEGAWA. There are numerous experiments[38,39] on the determination of 17-KS in diabetics, however, the results do not come to accordance, some insisting on an increase and some on a decrease.

The author studied 17-KS excretion after total pancreatectomy with gonadotropin administration at 4th, 8th and 12th week. Excretion of 17-KS increased slightly after total pancreatectomy until 4th week, which decreased from 8th to 12th week. YOSHIOKA reported that 17-KS decreased a week after the operation and increased to a level as high as 3 times of preoperative value, 2 or 3 weeks after the operation. However, this is the 17-KS excretion until 20th day and in the present experiment the increase was observed until 4th week and it was taken place by decrease thereafter. In determination of 17-KS in totally depancreatized dogs, response of long survivors is particularly concerned, and it is presumed that the function of the adrenal cortex and genital gland comes to decay after 4 weeks. AIKAWA reported that the adrenal cortex shows temporary hyperfunction about 2 weeks after total pancreatectomy, which is followed by gradual lowering thereafter. Finding in the present experiment also corresponded to his result.

Concerning blood sugar level after total pancreatectomy, many literatures have been published. According to TAKEDA[40] and KAWAMURA[41], preoperative level of 85 mg/dl arised to 306 mg/dl a day after total pancreatectomy. Insulin is used for this hyperglycemia and requirement of insulin provides some problems. DRAGSTEDT pointed out that insulin requirement markedly decreases as time elapses on after the operation and explained this phenomenon to be due to fatty liver caused by deficiency of antifatty liver hormone, *Lipocaic*. However, HONJO and YAO[42] investigated the relationship between liver glycogen content and assimilation rate and concluded that it was difficult to find certain correlation between these two. They further maintained that the result of insulin test in totally depancreatized dogs must be more closely controlled by the function of each endocrine system, than the influence of the liver. In the present experiment, blood sugar level was on the average 10mg/dl after an hour, 12mg/dl after 2 hours and 14mg/dl after 3 hours respectively lower in animals with gonadotropin administration than in those without it. Whereas in 2 cases of gonadotropin administration alone without insulin, fall of blood sugar level was not observed. In other words, it was observed that simultaneous administration of insulin and gonadotropin has more marked effect of lowering the blood sugar level than simple insulin administration. This fact is accepted to suggest that gonadotropin acts on the genital gland to increase estrogen and the increased estrogen acts on the anterior pituitary, at the same time influencing depressively against diabetogenic activity of the anterior pituitary, and on the other side, the effect of the increased estrogen and insulin are augumented to each other consequently lowering blood sugar level. The author observed marked lowering of blood sugar level and death of hypoglycemic shock in the both of 2 cases of totally depancreatized dogs received simultaneous administration of estrogen of 10000 u. every day with insulin.

Almost all cases of totally depancreatized dogs showed rapid emaciation and died in early period. This is interpreted to be due to disturbance of digestion and absorption caused by deficiency of exocrine function of the pancreas brought about by total pancreatectomy

and at the same time due to disturbance of glucose utilization and inability of adjustment of blood sugar level, which necessitates mobilization of depot fat, leading animals to emaciation. However, in the present experiment, body weight of animals with gonadotropin administration decreased slightly until 4th week, but after 4th week no change was observed in body weight, serum protein also remaining in the normal range and survived for long. In these animals diarrhea was not observed and feces were seemingly well digested. Slight decrease in body weight is thought to be due to this fact and above mentioned improvement of glucose utilization and consequent preserve of subcutaneous depot fat.

B. S. P. retention was 2.5% at 15th minute and there was no difference between animals with and without gonadotropin administration. At autopsy of long survivor which received gonadotropin administration, development of fatty liver was not observed macroscopically and subcutaneous depot fat was also well preserved.

In 1948, GYÖRGY[43] discovered that estradiol markedly decreases fat in the liver of albino rats of alimentary fatty liver, that is, antifatty liver effect of estradiol. As the inhibitory effect of estrogen on the anterior pituitary is ascertained by SPENCER[44], MEYER[45] and others, various experiment have been attempted concerning the relationship between the genital gland and diabetes. BARNES[46] and others reported that experimental diabetes could be improved by estrogen, and LEWIS[47] also reported that incidence of diabetes following partial pancreatectomy was inhibited by estrogen. On the other hand, however, INGLE[48] observed that incidence of diabetes following pancreatectomy increased by diethylstilbesterol. Concerning the influence of genital hormone on glucose metabolism in diabetics, it has been said in general that lutein hormone has no definite influence and follicle hormone increases glycosuria when administered in small dosis. COLLENS[49] reported that no favorable effect was observed when estrogen of 400 u. to 600 u. was administered in 7 cases (1 male and 6 females) of diabetics. MAZER and ISRAEL[50] suggested the necessity of administration of estrogen of as large dose as 10000 u. or more, based on the observation that slight elevation of blood sugar level and glycosuria was observed when estrogen of 2000 u. was administered every day in 51 cases of crimacterium (including 3 cases of diabetes). GESSLER observed favorable effect such as decrease in insulin requirement, disappearance of glycosuria and improvement of hyperglycemia by estrogen administration of more than 10,000 u. every day or every other day in diabetics of crimacterium. MORTON[51] reported a case who had diabetes since 12 years of age and showed hypoglycemic episode before menstruation and rapid hyperglycemia during or after it since her menarche, and change of blood sugar level became more intense since oophorectomy at the age of 30 years, and phase of glucose metabolism became stable, showing improvement of hyperglycemia, decrease in glycosuria decrease in insulin requirement and marked improvement of diabetic symptoms by 5 times, administration of estradiol propionate of 1 to 5 mg every other day. He concluded that estrogen is effective for diabetes which develops by excessively secreted hypophyseal hormone for compensation of ovarial hypofunction. These findings also correspond to the results of the present experiment. However, the fact that diabetes develops by estrogen as reported by COLLENS, MAZER, ISRAEL and others, is comprehended by the report of HOUSSAY that effect of steroid is sometimes observed in 2 phases. In other words, whether estrogen improves or develops diabetes depends upon the dosis of estrogen and the dosis must be further discussed as an important problem.

Proportion of ovarial weight to preoperative body weight was smaller than in control animals, suggesting hypofunction of the organ. Atresia folliculi and hyperplasia of interstitial tissue were also observed histologically. Accordingly, hypofunction of the organ was also ascertained by histological study. Histological picture corresponded to that reported by YOSHIOKA.

From these finding of the present experiment, estrogen level rapidly falls until 4 th week which is followed by further gradual fall thereafter, when determined in totally depancreatized dogs with gonadotropin administration. On the other hand, more pronounced decrease in blood sugar was observed by gonadotropin administration, and weight loss was not observed. This is presumed that while estrogen determination is not feasible in normal dogs due to extremely minute excretion, estrogen increases by gonadotropin administration and acts depressively on the anterior pituitary, at the same time inhibiting diabetogenic activity of the gland and consequently improving diabetic symptoms. Furthermore, another presumption may be justifiable, that is, this is not caused by direct effect of estrogen, but estrogen regulates genital function by way of the interbrain pituitary system and endocrine system restores its functional balance as a result of disappearance of interference of the genital gland or anterior pituitary against glucose metabolism regulating mechanism, further stabilizing glucose metabolic phase and accelerating curative effect of insulin.

Absence of weight loss is interpreted to be related, to some extent, to digestion and absorption, however, this must be further studied in the future.

V. Summary

Genital function of totally depancreatized dogs treated with insulin was studied under gonadotropin administration. Following results were obtained.

1) After total pancreatectomy, estrogen decreased rapidly until 4th week and decreased on gradually thereafter.

2) After total pancreatectomy, 17-KS increased until 4th week, but decreased thereafter.

3) Fall of blood sugar level caused by insulin was more pronounced in animals with gonadotropin administration than in those without it.

4) Serum protein decreased markedly in animals without gonadotropin administration, while it was mild in animals with it.

5) B. S. P. retention was less than 2.5% at 15th minute in both animals with and without gonadotropin administration.

6) Marked weight loss was observed in animals without gonadotropin administration, while weight loss was hardly observed in animals with it.

7) Proportion of ovarial weight to preoperative body weight was smaller than in control animals.

8) Histological finding revealed hypofunction of the ovarium compared with control animals.

9) From these findings it was ascertained that function of the genital gland comes to decay after total pancreatectomy, on the other hand, estrogen improves diabetes.

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(* in Japanese)

Plate 1. Ovarium of Normal Dog. ×100 (H-E)

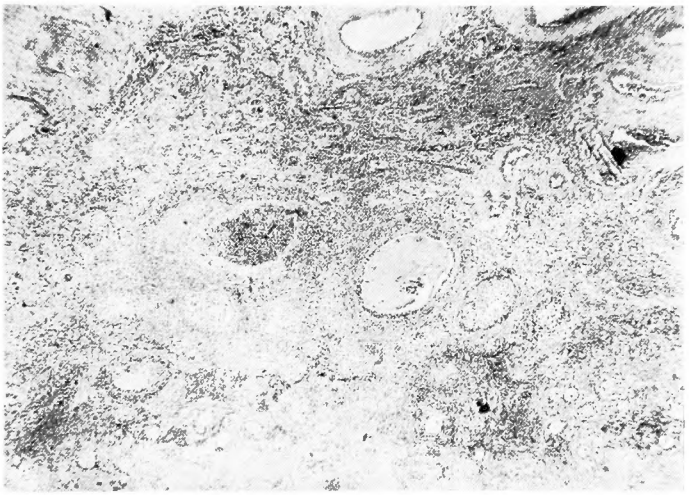

Plate 2. Ovarium of Totally Depancreatized Dog with Administration of Insulin and Gonadotropin, 101 Days after Operation. ×100 (H-E)

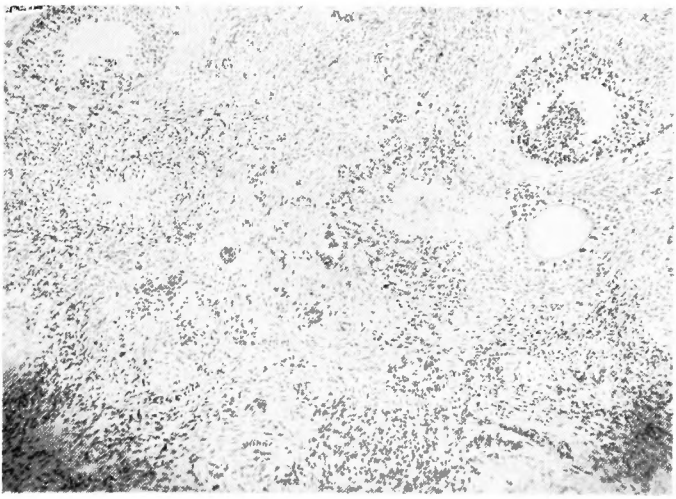

Plate 3. Ovarium of Totally Depancreatized Dog with Administration of Insulin and Gonadotropin, 158 Days after Operation. ×100 (H-E)

和文抄録

インシュリン投与下に於ける膵全剔雌犬のEstrogenの態度

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西川真澄

インシュリン投与下の膵全剔雌犬の性腺機能が如何になるかをGonadotropinを投与して検索し，次の結果を得た．

1) 膵全剔後Estrogenは，4週迄に急激な低下を辿るが，4週以後は徐々に低下した．

2) 膵全剔後17-KSは，4週迄増加を示すが，4週以後は低下した．

3) 血糖値は，Gonadotropin無投与群より，Gonadotropin投与群は，インシュリン投与に対する血糖降下が著明であつた．

4) 血清蛋白値は，Gonadotropin無投与群は著しい減少を来たすのに反し，Gonadotropin投与群は減少が少なかつた．

5) BSP値は，無投与群，投与群共に15分値2.5%以下であつた．

6) 体重は，Gonadotropin無投与群は著しい体重減少を認めたが，Gonadotropin投与群は体重減少がほとんど認められなかつた．

7) 卵巣重量は，術前体重に比較せる卵巣の比体重重量は，対照犬に比し減少していた．

8) 組織学的検索では，正常犬に比して術後像は機能低下の像であつた．

以上の結果から膵全剔後の性腺機能は低下を示すが，一方Estrogenの作用は糖尿病を軽減する事を認めた．